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Research Article

Two new species of the genus *Conocybe* (Agaricales, Bolbitiaceae) from Punjab, Pakistan

Yilan Jin[1], Muhammad Haqnawaz[2], Abdul Rehman Niazi[2], Pingzhu Lu[3], Abdul Nasir Khalid[2], Qirui Li[4]

1 *Agricultural College, Anshun University, Anshun, Guizhou Province, 561000, China*
2 *Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Quaid–e–Azam Campus 54590, Lahore, Pakistan*
3 *Chongqing Three Gorges Medical College, Wanzhou Chongqing, 404100, China*
4 *The High Efficacy Application of Natural Medicinal Resources Engineering Centre of Guizhou Province (The Key Laboratory of Optimal Utilization of Natural Medicine Resources), School of Pharmaceutical Sciences, Guizhou Medical University, Guiyang, Guizhou, 550004, China*

Corresponding authors: Muhammad Haqnawaz (m.haqnawaz144@gmail.com); Qirui Li (lqrnd2008@163.com)

Abstract

In this study, two new species of genus *Conocybe* are described, based on morpho-anatomical and phylogenetic analyses of ITS and LSU (28S) regions. According to phylogenetic analyses, *Conocybe kotadduensis* & *C. safariensis* make a separate clade with a strong bootstrap value from the closest species of the genus *Conocybe*, sect. *Pilosellae*. *Conocybe kotadduensis* is closest to *C. nitrophila*, while *C. safariensis* is closer to *C. ceracea*. *Conocybe kotadduensis* differs from *C. nitrophila* due to pale yellow, parabolic pileus with velvety surface, scabrous surface of stipe, amygdaliform to ovoid, yellowish basidiospores, broad, fusiform, cylindrical cells of lecythiform cheilocystidia, sphero-pedunculate to obovoid, globose to sub-globose pileipellis elements. *Conocybe safariensis* can be distinguished from *C. ceracea* in having a conical to convex pileus, orange to light gray stipe, fusiform to lacrymoid basidiospores, obovoid to subglobose basidia, ellipsoid to globose cheilocystidia, and absence of pleurocystidia.

Key words: Basidiomycetes, macrofungal diversity, mushrooms, phylogenetic analysis, Pilosellae, taxonomy

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Introduction

Genus *Conocybe* Fayod (Bolbitiaceae), is widely distributed in the world and mostly found in temperate regions of Asia, on herbivorous dung, fertile soil, grassland, and lawns etc. (Hausknecht 2009; Song and Bau 2023). It is a toxic fungus that contains toxic substances, such as phallotoxins, psilocybin, and amatoxins, which cause health problems like liver damage, gastroenteritis, and neuropsychological problems (Wu et al. 2019; Song and Bau 2023; Song and Bau 2025). However, these toxic substances also have shown significant pharmacological activities, such as treatment-resistant depression and post-traumatic stress disorder (Griffiths et al. 2016; Song and Bau 2023; Song and Bau 2025). It is characterized by a conical pileus, brown-rusty lamellae, and slender stipe often covered with powdery or hairy, lecythiform cheilocystidia (Song and Bau 2023; Song and Bau 2025). The genus *Conocybe* consist of 12 sections, viz., *Conocybe* sect. *Candidae*

Watling, sect. *Conocybe* Fayod, sect. *Conocybella* (Singer) Watling, sect. *Gigantea*e Singer, sect. *Heterocystidiae* E.F. Malysheva, sect. *Inopinatae* Hauskn. & Contu, sect. *Mixtae* Singer, sect. *Nodulososporae* Watling, sect. *Obscurae* Hauskn. & Krisai, sect. *Ochromarasmius* (Singer) Hauskn. & Krisai, sect. *Pilosellae* Singer, and sect. *Singerella* Watling (Hausknecht and Contu 2007; Hausknecht and Krisai-Greilhuber 2007; Malysheva 2017; Song and Bau 2023; Asif et al. 2025). Our two newly identified taxa belong to sect. *Pilosellae*, distinguished by an incomplete white stipe, numerous hairs, and non-lecythiform caulocystidia, with *C. pilosella* as the type species. From Pakistan, only 14 species have been previously reported (Akram et al. 2025; Asif et al. 2025). The aim of this work is to generalize the current knowledge about species diversity of this genus and to provide the detailed descriptions of the species collected from the Pakistan.

Materials and methods

Sampling and study area (Fig. 1)

Specimens were collected during Jun-November 2020–2023, in Bet Faqirwali and Safari Park, bed of the Indus River, District Kot Addu, (30°14'44"N, 70°51'04"E, 134 m a.s.l.), Punjab Province, Pakistan. The climate of Kot Addu is very hot during summer and mild in winter with an approximate highest and lowest temperature of 51 °C and -1 °C respectively; average annual rainfall is 127 mm (Haqnawaz et al. 2023a, 2024a, b; Li et al. 2025). Vegetation of the sampling site is dominated by subtropical plant species such as *Albizia chinensis* (Osbeck) Merrill *Calotropis procera* (Ait.) Ait., Hort., *Dalbergia sissoo* Roxb., *Mangifera indica* L. *Saccharum bengalense* Retz., *S. officinarum* L., *Tamarix aphylla* (L.) Warb., and *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb, (Stewart 1972; Haqnawaz et al. 2023a, b). Samples were photographed, tagged, and then dried in front of the electric fan heater at about 35 °C temperature and were deposited in the Lahore Herbarium (LAH), Institute of Botany, University of the Punjab, Lahore, Pakistan.

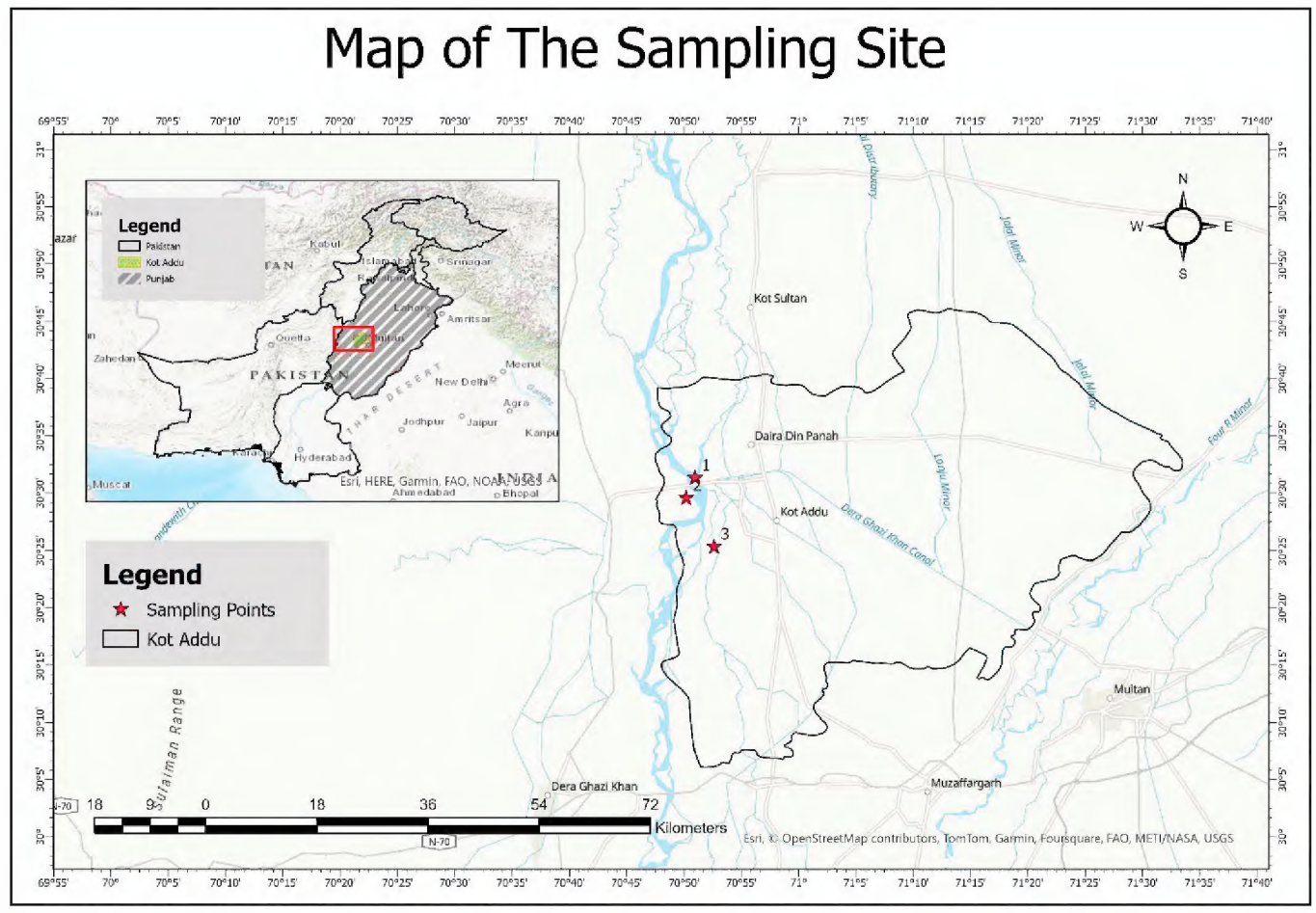

Figure 1. Maps of sampling sites where the novel taxa were found, shows with the red color.

Morpho-anatomical characterization

Macroscopic characteristics such as size, shape, and color of pileus and stipe were noted, based on fresh samples. Description terminology by Vellinga (2001) was followed, while for color determination, the Munsell color chart was followed (Munsell 1994). Congo red was used for dyeing. The microscopic features like size, shape, and color of basidiospores, basidia, cheilocystidia, pileipellis, and caulocystidia were observed using a compound microscope (OLYMPUS BX43, Tokyo, Japan), and measurements were taken using calibrated Piximètre software connected to a compound microscope through a HDCE-X5 microscopic camera. In the case of basidiospores, at least 50 spores were measured. The formula [n/m/p] indicates 'n' the number of basidiospores measured from 'm' basidiomata of 'p' collection. Basidiospores measurements are presented as (a–) b–c (–d), where b–c indicates 90% of the calculated values, while values in parenthesis are extreme lowest or extreme highest values; Q indicates the individual spore length/width ratio while Qav. is the average length/width ratio of all basidiospores (Ge et al. 2010).

Molecular phylogenetic analyses

DNA was extracted from the dried specimens using the 2% CTAB method (Bruns 1995). Primers used during amplifications were ITS1F 5'-CCT GGT CAT TTA GAG GAA GTA A-3' as forward and ITS4 5'-TCC TCC GCT CTA TTG ATA TGC-3' as reverse for nrITS region (>775 bp) while LROR 5'-ACC CGC TGA ACT TAA GC-3' as forward and LR5 5'-TCC TGA GGG AAA CTT CG-3' as reverse for nrLSU region (>910 bp) (Gardes and Bruns 1993, White et al. 1990). The Polymerase Chain Reaction (PCR) was performed in a 50 µl reaction volume: containing 5 µl 10× Econo Taq Buffer (Lucigen, Middleton, Wisconsin, USA), 1 µl dNTPs, 2.5 µl of each primer (10 µM/µl), 0.25 µl of Econo Taq® DNA Polymerase (Lucigen), 28.75 µl H_2O and 10 µl DNA template. All PCR amplifications were carried out with the cycling program for 35 cycles as follows, denaturation at 94 °C for 30 sec, annealing at 54 °C for 1 min, extension at 71 °C for 2 min, with an initial denaturation at 94 °C for 2 min and a final extension of 71 °C for 5 min. PCR products were then sequenced and analyzed (Usman and Khalid 2020; Haqnawaz et al. 2023b).

Forward and reverse primer reads of both ITS and nrLSU regions were combined and consensus sequences were obtained using Bioedit. ver. 7.2.5 (Hall 1999). The resulting sequences were subjected to a BLAST search at NCBI (https://www.ncbi.nlm.nih.gov/guide/). The sequences of closely related species from GenBank and sequences used from a recent publication of *Conocybe*, were included in our final phylogenetic tree (Asif et al. 2025; Song and Bau 2025). One sequence of *Bolbitius reticulatus* (Pers.) Ricken was used as an outgroup for the ITS and LSU datasets. For the phylogenetic analyses, a Clustal W MUSCLE alignment was implemented in BioEdit v. 7.2.5 with manual adjustments. A combined (ITS–LSU) maximum likelihood phylogenetic tree was constructed using RAxML-HPC2 v. 8.2.12 on XSEDE (8.2.10) implemented on the CIPRES Science Gateway (Miller et al. 2010). The GTR+GAMMA nucleotide substitution model was used, with 1,000 bootstrap iterations performed using rapid bootstrapping. The resulting bootstrap values were then mapped on to the best-scoring ML tree and show in Fig. 2. The model of evolution was estimated by MrModeltest 2.2 (Nylander 2004). Markov chain Monte Carlo (MCMC) sampling in MrBayes v.

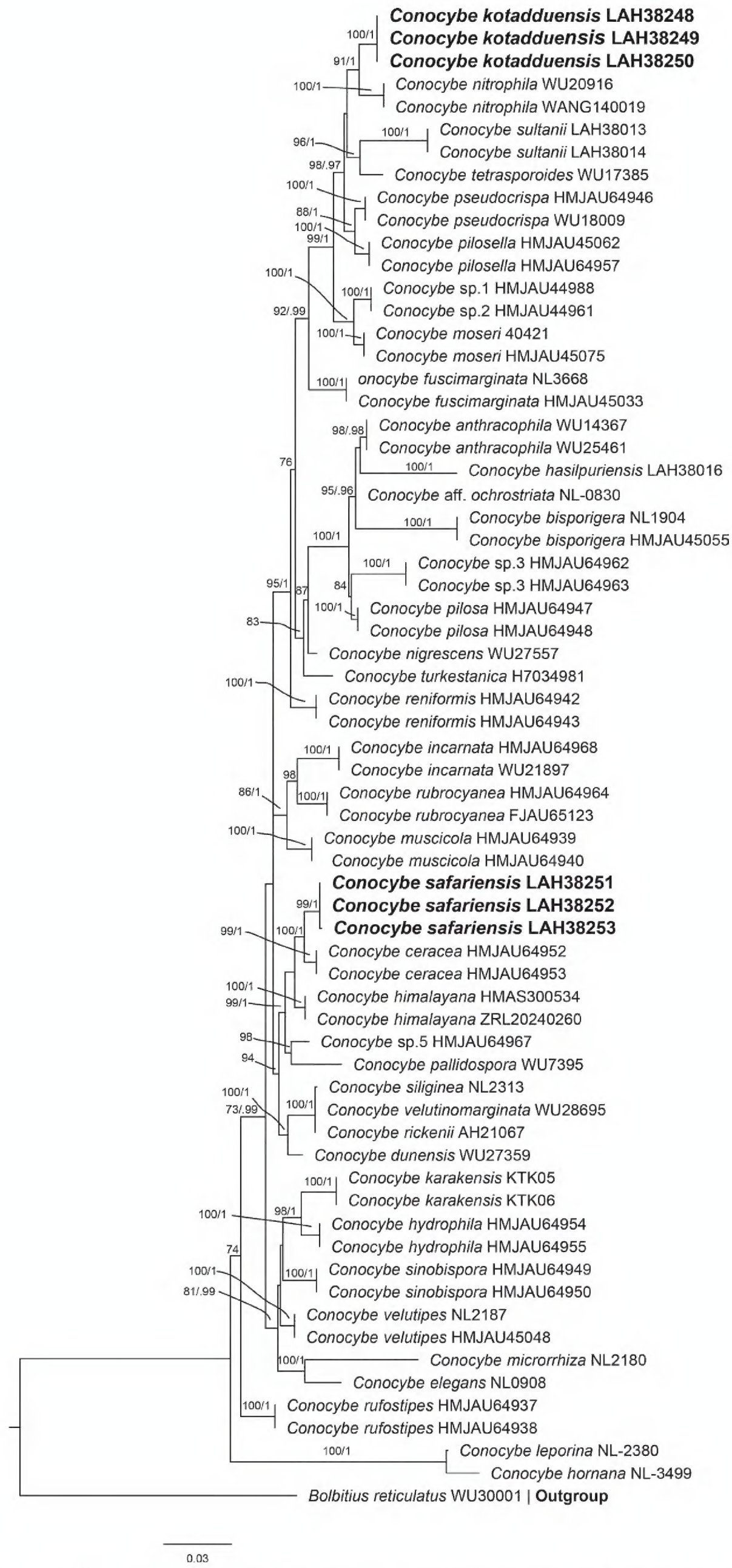

Figure 2. Phylogenetic tree of the genus *Conocybe* as generated by maximum likelihood (ML) and Bayesian analyses, based on combined ITS and LSU sequences. Bootstrap values ≥ 50%, based on 1,000 replicates and Bayesian posterior probabilities (PP) ≥ 0.90, are shown at the branches. Novel sequences, generated during this study, are shown in bold.

3.2.2 (Ronquist et al. 2012) was used to determine posterior probabilities (PP). Six simultaneous Markov chains were run for 1,000,000 generations, and trees were sampled every 1,000th generation. Bootstrap values ≥ 50% and Bayesian PP ≥ 0.90 are indicated on the branches, which were visualized in FigTree v. 1.4.2 (Rambaut 2014). The newly generated sequences were submitted to GenBank, and short descriptions of the species were deposited in MycoBank (Robert et al. 2013). Boot strap values ≥ 50% are mentioned on the branches that were visualized in FigTree v. 1.4.2 (Rambaut 2014). The newly generated sequences were deposited in GenBank, and short descriptions of the species were deposited in MycoBank (Robert et al. 2013). All the sequences used in the final phylogenetic tree are presented in Table 1, together with voucher numbers, GenBank accession numbers, and country of origin. The newly generated sequences were deposited in GenBank and are in bold in the phylogenetic trees and Table 1.

Results

ITS & LSU phylogenetic analyses (Fig. 2, Table 1)

The combined phylogenetic tree of the nrITS & nrLSU region based on consists of 65 sequences, and *Bolbitius reticulatus* (WU30001) is an outgroup. Both new taxa are shown in bold, and formed separate clades from their closely related species. *Conocybe kotadduensis* formed a separate branch with 78% bootstrap support from its sister species, *Conocybe nitrophila* (Hauskn.) Yen W. Wang & S.S. Tzean (WU20916 & WANG140019); and the other closest species is *Conocybe pilosella* (Pers.) Kühner (HMJAU45062 & HMJAU64957). The second new species *Conocybe safariensis* also formed a separate branch from its sister species *C. ceracea* T. Bau & H.B. Song (HMJAU64952 & HMJAU64953) with high bootstrap value 94%, and *C. himalayana* Ke Wang, T.Z. Wei & P. Hong (HMAS300534 & ZRL20240260) with 89% bootstrap value.

Taxonomy

***Conocybe kotadduensis* Haqnawaz, Niazi & Khalid, sp. nov.**
MycoBank No: 855941
Figs 3, 4

Etymology. Species name "*kotadduensis*" (Latin) refers to the type locality Kot Addu, Punjab, Pakistan.

Holotype. PAKISTAN • Punjab, Kot Addu District, bed of Indus River, (30°23'27"N, 70°48'28"E, 127 m a.s.l.), on plant debris, June 14, 2023, *Muhammad. Haqnawaz*, KA-06 (LAH38248). GenBank: PQ409233 (nrITS), PQ409236 (nrLSU).

Diagnosis. *Conocybe kotadduensis* is different from the closest species, *C. nitrophila*, by its relatively light gray to pale yellow, parabolic absence of thick glutinous veil and viscid in regular to slightly crenate margins of pileus, adnexed, broad, thick lamellae with 2–3 tires of lamellulae, scabrous surface of stipe, amygdaliform to ovoid basidiospores, mostly broad bi-sterigmate of basidia, clavate, utriform with median constriction, fusiform, cylindrical shape of lecythiform cheilocystidia, sphero-pedunculate to obovoid, globose to sub-globose pileipellis elements.

Table 1. Fungal species used for phylogenetic analyses of *Conocybe* including their species names, locality, voucher and GenBank accession numbers of the nrITS & nrLSU regions. Sequences generated for this study are shown in bold.

Species names	Locality	Voucher	GenBank No.	
			ITS	LSU
Conocybe anthracophila	Italy	WU25461	JX968237	JX968355
Conocybe anthracophila	Italy	WU14367	JX968212	JX968329
Conocybe aff. *Ochrostriata*	Hungary	NL-0830	JX968236	JX968354
Conocybe bisporigera	China	HMJAU45055	OP526418	-
Conocybe bisporigera	Hungary	NL-1904	JX968235	JX968353
Conocybe ceracea	China	HMJAU64952	OQ758111	OQ758219
Conocybe ceracea	China	HMJAU64953	OQ758112	OQ758220
Conocybe dunensis	Spain	WU27359	JX968227	JX968345
Conocybe elegans	Sweden	NL-0908	JX968223	JX968341
Conocybe fuscimarginata	Sweden	NL-3668	JX968238	JX968356
Conocybe fuscimarginata	China	HMJAU45033	OQ780310	OQ758208
Conocybe hasilpuriensis	Pakistan	LAH38016	PP817194	OR773176
Conocybe hornana	Slovakia	NL-3499	JX968178	JX968294
Conocybe himalayana	China	HMAS 300534	PQ099842	PP968799
Conocybe himalayana	China	ZRL20240260	PQ699279	PQ699303
Conocybe hydrophila	China	HMJAU64954	OQ758116	OQ758232
Conocybe hydrophila	China	HMJAU64955	OQ758117	OQ758233
Conocybe incarnate	China	HMJAU64968	OQ780316	-
Conocybe incarnate	Finland	WU21897	JX968229	JX968347
Conocybe juniana	Sweden	NL-2105	JX968191	JX968307
Conocybe karakensis	Pakistan	KTK05	ON392730	-
Conocybe karakensis	Pakistan	KTK06	ON392731	
Conocybe kotadduensis	**Pakistan**	**LAH38248**	**PQ409233**	**PQ409236**
Conocybe kotadduensis	**Pakistan**	**LAH38249**	**PQ409234**	**PQ409237**
Conocybe kotadduensis	**Pakistan**	**LAH38250**	**PQ409235**	**PQ409238**
Conocybe leporina	Hungary	NL-2380	JX968177	JX968293
Conocybe microrrhiza	Hungary	NL-2180	JX968222	JX968340
Conocybe moseri	Germany	40421	MK412354	-
Conocybe moseri	China	HMJAU45075	OQ780309	OQ758207
Conocybe muscicola	China	HMJAU64939	OQ758113	OQ758223
Conocybe muscicola	China	HMJAU64940	OQ758114	OQ758224
Conocybe reniformis	China	HMJAU64942	OQ758108	OQ758229
Conocybe reniformis	China	HMJAU64943	OQ758109	OQ758228
Conocybe nigrescens	Italy	WU27557	JX968234	JX968352
Conocybe nitrophila	India	WU20916	JX968233	JX968351
Conocybe nitrophila	China	WANG140019	KR998384	-
Conocybe pallidospora	Austria	WU7395	JX968239	JX968357
Conocybe pilosa	China	HMJAU64947	OQ758122	OQ758222
Conocybe pilosa	China	HMJAU64948	OQ758123	OQ758221
Conocybe pilosella	China	HMJAU45062	OQ780305	OQ758205
Conocybe pilosella	China	HMJAU64957	OQ780306	OQ758206
Conocybe pseudocrispa	China	HMJAU64946	OQ780307	OQ758212
Conocybe pseudocrispa	Austria	WU18009	JX968230	JX968348
Conocybe rickenii	Spain	AH21067	MF142238	-
Conocybe rubrocyanea	China	FJAU65123	PP501388	PP501398
Conocybe rubrocyanea	China	HMJAU64964	PP501389	PP501399
Conocybe rufostipes	China	HMJAU64937	OQ758120	OQ758227
Conocybe rufostipes	China	HMJAU64938	OQ758121	OQ758226
Conocybe safariensis	**Pakistan**	**LAH38251**	**PP973138**	**PP973144**

Species names	Locality	Voucher	GenBank No.	
			ITS	LSU
Conocybe safariensis	**Pakistan**	**LAH38252**	**PP973139**	**PP973145**
Conocybe safariensis	**Pakistan**	**LAH38253**	**PP973140**	**-**
Conocybe siliginea	Swedan	NL-2313	JX968225	JX968343
Conocybe sinobispora	China	HMJAU64949	OQ758118	OQ758230
Conocybe sinobispora	China	HMJAU64950	OQ758119	OQ758231
Conocybe sp. 1	China	HMJAU44988	OQ749737	OQ740305
Conocybe sp. 2	China	HMJAU44961	OQ749738	-
Conocybe sp. 3	China	HMJAU64962	OQ749739	OQ740306
Conocybe sp. 4	China	HMJAU64963	OQ749740	OQ740307
Conocybe sp. 5	China	HMJAU64967	OQ749741	-
Conocybe sultanii	Pakistan	LAH38013	OR773181	OR773183
Conocybe sultanii	Pakistan	LAH38014	OR773180	OR773182
Conocybe turkestanica	Turkestan	H7034981	H7034981	MH055382
Conocybe tetrasporoides	New Zealand	WU17385	JX968232	JX968350
Conocybe velutinomarginata	Germany	WU28695	JX968226	JX968344
Conocybe velutipes	Hungary	NL-2187	JX968228	JX968346
Conocybe velutipes	China	HMJAU45048	OQ780311	OQ758209
Bolbitius reticulatus ***Outgroup***	Hungary	WU30001	JX968249	JX968366

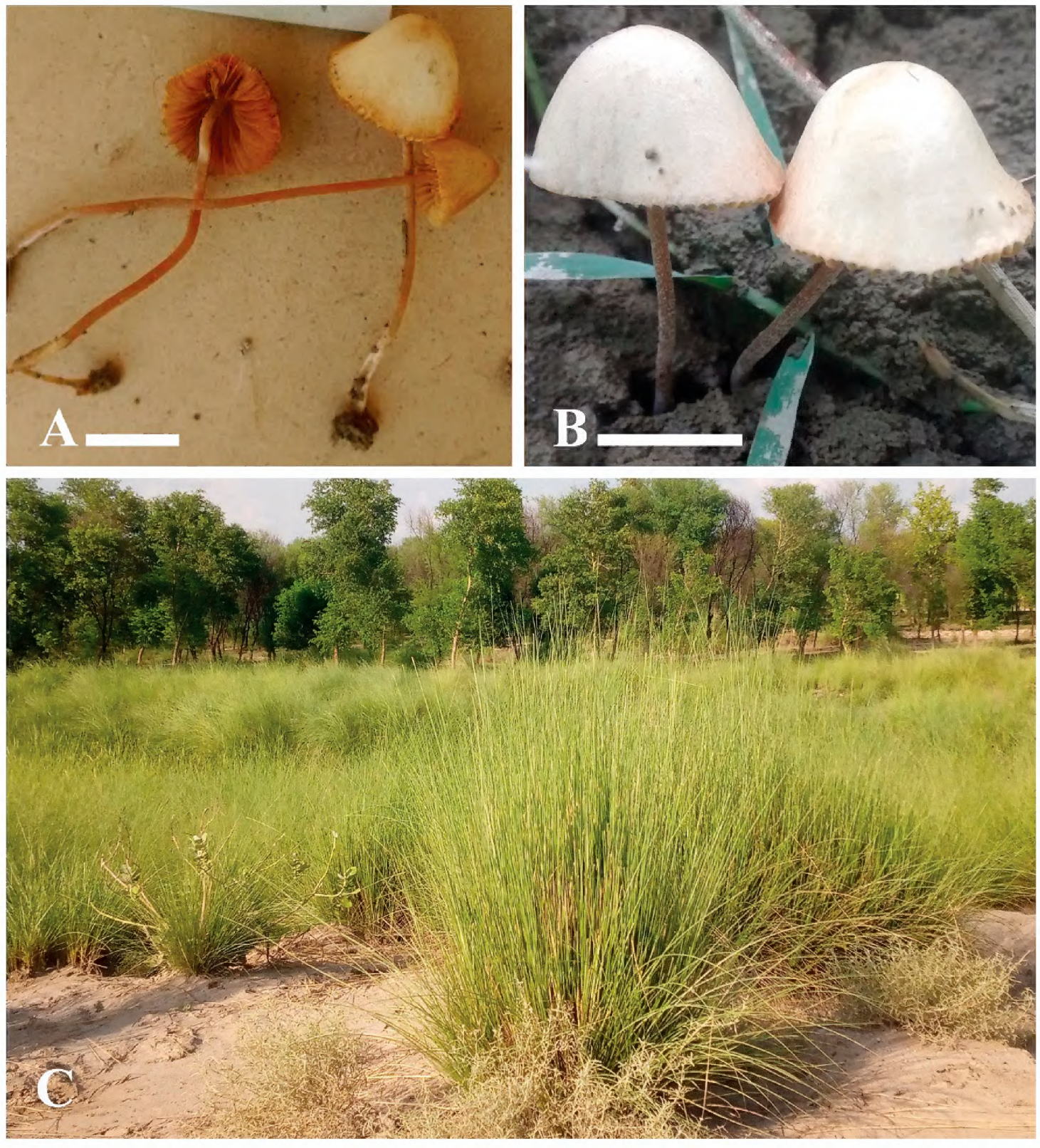

Figure 3. A, B. Basidiomata of *Conocybe kotadduensis*. (**A.** Holotype LAH38248); **C.** Sampling site. Scale bars: 10 mm (**A, B**).

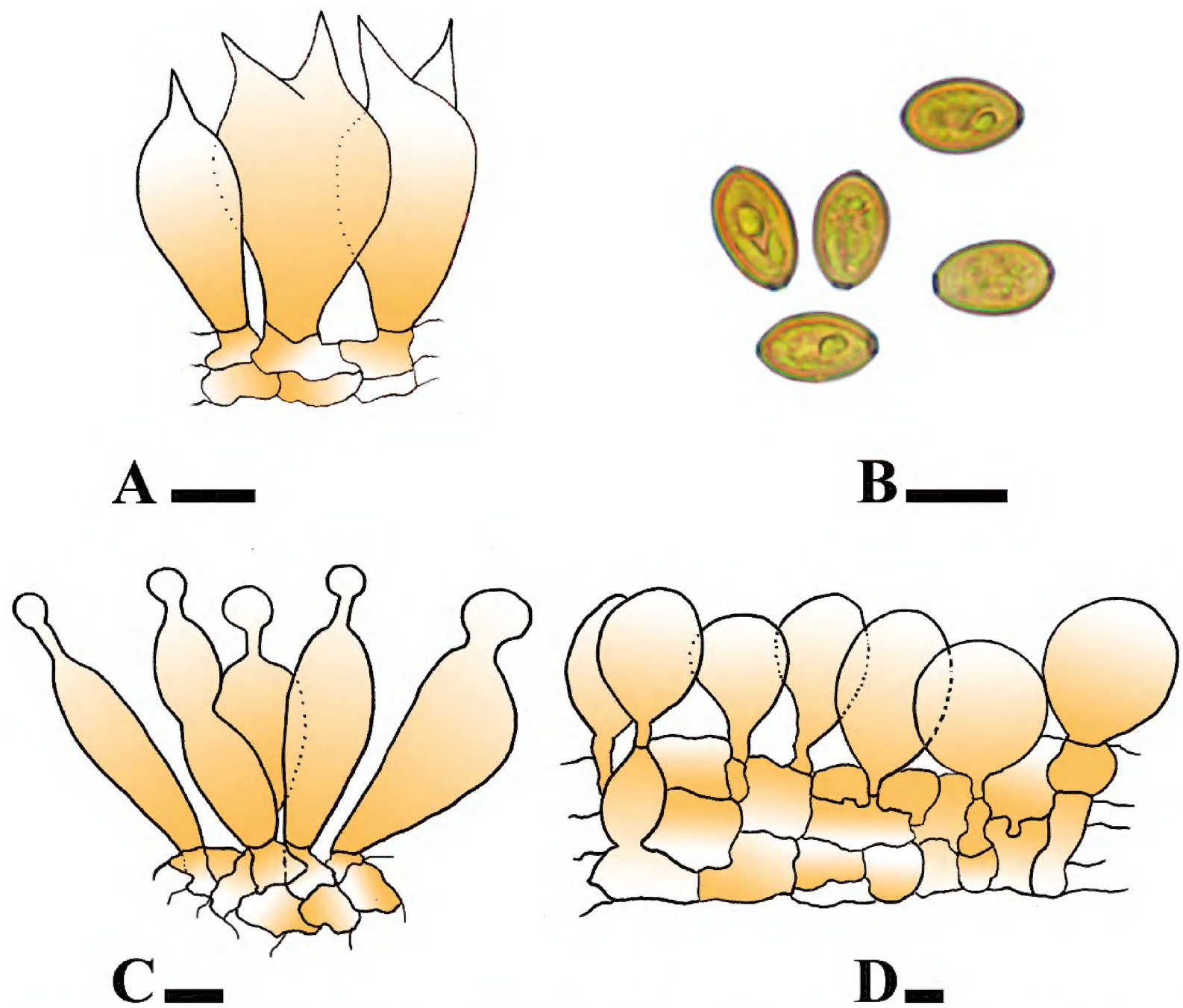

Figure 4. A–D. Microscopic structures of *Conocybe kotadduensis*. (**A.** Holotype LAH38248). **A.** Basidia; **B.** Basidiospores; **C.** Cheilocystidia; **D.** Pileipellis elements. Scale bars: 10 µm.

Description. ***Pileus*** 10–13 mm diam., hemispherical to parabolic at young stage, conical, parabolic when old, straight shape of margins, regular to slightly crenate cap margins, velutinous surface, light gray (7.5 YR 8/1) to pale yellow (2.5 Y 8/4), with dull yellow orange (10YR 7/4) center. ***Lamellae*** adnexed, broad, thick, subdistant, fimbriate, unequal, with exceeding 1–3 tires, dull yellow orange (10 YR 7/4) to orange (7.5 YR 7/6). ***Stipe*** 25–49 mm in length, cylindrical, flexuous, central, with slightly scabrous surface, hard and dry, slightly broad toward base, orange (5 Y.R 6/6) at upper side, dull yellow orange (10 YR 7/4) at base, grayish white (N 8/0).

Basidiospores [100/2/3], (13.2–)13.5–15.5 (–16) × (8–)8.5–9.5(–10) µm, avl × avw = 14.7 × 9.0 µm, Q = 1.47–1.69, Q_{av} = 1.62, amygdaliform to ovoid, yellowish to slightly light brown in 5% KOH, thin walled, guttulate, prominent germ pore. ***Basidia*** [30/2/3] (24–)24–28(–29) × (10.5–)11–11(–11.5) µm, clavate, with 2 sterigmata with broad width, hyaline in 5% KOH, thin-walled. ***Cheilocystidia*** [30/3/3] (14–) 14.5–24(–24.5) × (5.5–)6.–10.5(–11) µm, lecythiform with clavate, utriform with median constriction, fusiform, cylindrical shap of cells, rarely lageniform, hyaline, thin walled. ***Pileipellis*** [30/2/3] (25–89 × 1637 µm, sphero-pedunculate to obovoid, globose to sub-globose, hyaline and thin walled. ***Stipitipellis*** 8–23 µm, avw = 9.8 µm, cutis, hyaline in 5% KOH, thin walled, septate, unbranched, regular. ***Caulocystidia*** [15/3/3] 9–14 × 5–7 µm, rarely present, clavate to cylindrical, hyaline in 5% KOH, thin walled. Clamp connection absent.

Ecology and habitat. Gregarious, terrestrial, under plants of *Saccharum bengalense* and *Cyperus rotundus*.

Additional specimens examined. PAKISTAN • Punjab, Kot Addu, bed of Indus River, 30°25'19"N, 70°52'44"E, 132 m a.s.l., June 26, 2020, FW-98, *Muhammad Haqnawaz*, (***Paratype*** LAH38249), GenBank: PQ409234 [nrITS], PQ409237 [nrLSU]. • 30°14'44"N, 70°51'04"E, 134 m a.s.l. under *Cyperus rotundus*, August 15, 2021, TA-51, Muhammad Haqnawaz (***Paratype*** LAH38250), GenBank: PQ409235 (nrITS), PQ409238 (nrLSU).

Conocybe safariensis **Niazi, Haqnawaz & Khalid, sp. nov.**
MycoBank No: 854578
Figs 5, 6

Etymology. Species name *safariensis* (Latin) refers to the locality of the taxon. i.e., Safari Park, Kot Addu, Punjab, Pakistan.

Holotype. PAKISTAN • Punjab, Kot Addu District, bed of Indus River, (30°31'33.63"N, 70°56'29.71"E, 135 m a.s.l.), on plant debris under *Tamarix aphylla*, July 05, 2021, *Muhammad. Haqnawaz*, HQ-69 (LAH38252). GenBank: PP973139 (nrITS), PP973145 (nrLSU).

Diagnosis. *Conocybe safariensis* is different from the closest species, *C. ceraceа*, by its hemispherical, lentiform, obtusely conical, then convex to plane and straight margin of pileus, orange to light gray stipe, obovoid to lacrymoid basidiospores, obovoid, ellipsoid to globose cheilocystidia, oblong, globose, clavate and fusiform elements of pileipellis, ellipsoid to broadly ellipsoid, clavate to broadly clavate caulocystidia, and absence of pleurocystidia.

Description. ***Pileus*** 15–24 mm long, hemispherical to campanulate at young stage, sub hemispherical, lentiform, obtusely conical, then convex to plane when old, slightly regular, straight shape and sulcate cap margins, smooth, light gray to dull orange (7.5 YR 8/2, 7/4), texture soft, thin, light gray (7.5 YR 8/2), ***Lamellae*** adnate, broad, average, sub distant, even, unequal, exceeding, and 3–7 tires, orange (7.5 YR 7/6). ***Stipe*** 25–49 mm in length, flexuous, white scabrous surface, costate surface, bulbous base, orange (7.5 YR 4/3) at the base, light brownish gray (7.5 YR 7/2) to light gray (5 YR 8/1) toward base.

Basidiospores [100/2/3], (8–) 8.6–10 (–10.5) × (4.5–) 4.8–5.5(–6) µm, avl × avw = 9.5 × 5.2 µm, Q = 1.63–2.2, Qav = 1.8, obovoid to lacrymoid, yellowish green to light reddish brown in water, guttulate, thick walled, prominent germ pore present. ***Basidia*** [30/2/3] (15–)16–23(–24) × (7–)8–12(–13) µm, clavate to broadly clavate, slightly cylindrical, with 1–4 sterigmata, hyaline in 5% KOH, amyloid, thin–walled, guttulate. ***Cheilocystidia*** [40/2/3] 16–25 × 8–12 µm, lecythiform with ellipsoid to broadly ellipsoid, globose to subglobose, clavate to broadly clavate cells, hyaline, thick walled. ***Pleurocystidia*** absent. ***Pileipellis*** [30/2/3] 20–50 × 18–36 µm, irregular epithelium, oblong, globose to subglobose, clavate to broadly clavate, fusiform elements, thin-walled. ***Stipitipellis*** 8–17 µm, avw = 12.5 µm, regular, trichoderm, hyaline, thin walled, septate, unbranched, regular, clamp connection present. ***Caulocystidia*** [30/2/3] 11–33 × 7–13 µm, rearly present, cylindrical ellipsoid to broadly ellipsoid, globose to subglobose, clavate to broadly clavate cells hyaline in 5% KOH.

Ecology and habitat. Gregarious, terrestrial, under *Tamarix aphylla*, on loamy soil rich in organic matter.

Figure 5. A. Basidiomata of *Conocybe safariensis*. (**A.** Holotype LAH38252); **B.** Sampling site, Scale bar: 10 mm (**A**).

Additional specimens examined. PAKISTAN • Punjab, Kot Addu, Noor Shah Thal, 30°31'33.63"N, 70°56'29.71"E, 135 m a.s.l., September 29, 2022, *Muhammad Haqnawaz*, (***Paratype*** LAH38251), GenBank: PP973138 [nrITS], PP973144 [nrLSU]. • 30°25'21"N, 70°52'46"E 132 m. a.s.l., September 10, 2023, MQ-151, Muhammad Haqnawaz (***Paratype*** LAH38253), GenBank: PP973140 (nrITS).

Discussion

Two new species, *Conocybe kotadduensis* and *C. safariensis*, were described based on phylogenetic analyses and morpho-anatomical descriptions. Phylogenetic analysis of nrITS and nrLSU sequence data showed that

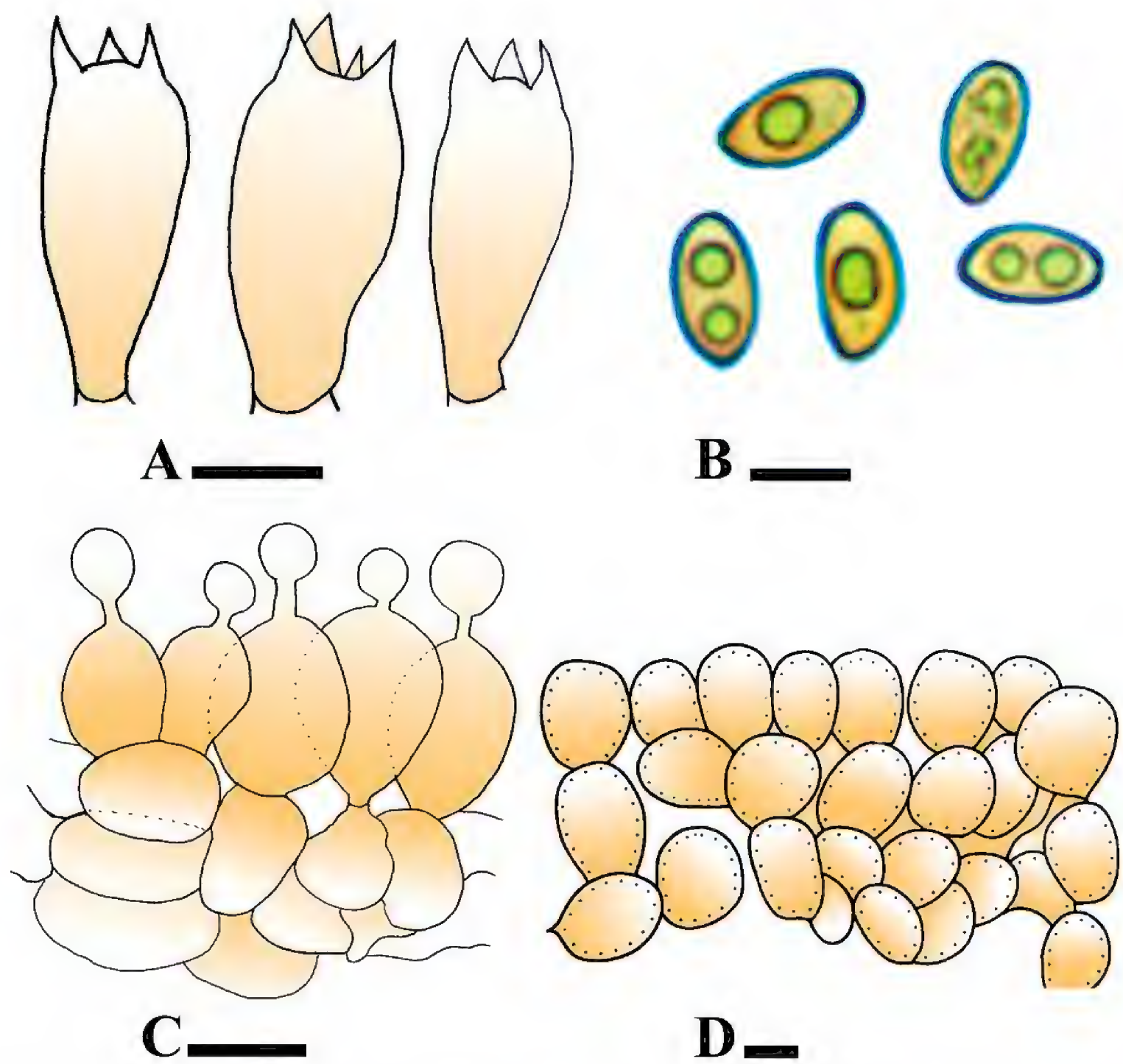

Figure 6. A–D. Microscopic structures of *Conocybe safariensis*. (**A.** Holotype LAH38252). **A.** Basidia; **B.** Basidiospores; **C.** Cheilocystidia; **D.** Pileipellis. Scale bars: 10 µm (**A–D**).

Conocybe kotadduensis and *C. safariensis* belong to section *Pilosellae* Singer (Song and Bau 2023). *Conocybe kotadduensis*, formed a separate branch from *C. nitrophila*, *C. pilosella*, and *C. pseudocrispa* with 18, 25, and 21 base pairs differences, respectively. While *Conocybe safariensis* is different from its sister's species, *C. ceracea*, *C. himalayana* and *C. pallidospora* with 14, 19, and 38 base pairs differences, respectively.

Morpho-anatomically, *Conocybe kotadduensis* is distinguished from its sister species, *C. nitrophila*, by having pale yellow, parabolic pileus with velvety surface (vs. orange white, hygrophanous, hemispherical to a little convex pileus with smooth surface), scabrous surface of stipe (vs. smooth), amygdaliform to ovoid, yellowish to slightly light brown basidiospores (vs. broadly ellipsoid to oval, brownish orange to reddish brown), broad, fusiform, cylindrical cells of lecythiform cheilocystidia (vs. only clavate shape of cells), sphero-pedunculate to obovoid, globose to sub-globose pileipellis elements (vs. only broadly cylindrical to clavate). *C. nitrophila* has thick glutinous veil and viscid in fresh sample while absence in new taxon (Wang and Tzean 2015). The second closer species, *Conocybe pilosella* differs in having subpilose pileus covered with hair, tetra-sterigmate basidia, absent germpore, lecythiform pileocystidia (Song and Bau 2023). Another species, *C. pseudocrispa*, is different due to its oyster white surface with green beige to ivory center of pileus, narrowly adnate, beige-brown

to ochre-brown lamellae, longitudinally striated, fine hairs, thick base of stipe, honey yellow to ochre-brown, ellipsoid to oblong basidiospores, subglobose, lageniform, lanceolate stipitipellis elements (Song and Bau 2023).

Similarly, morpho-anatomically, *Conocybe ceracea* differs from our second new taxon, *C. safariensis*, possessing subglobose, paraboloid to subcylindrical pileus, incurved margins, and presence of precipitate wax crystals on the surface of the pileus, honey yellow to melon yellow, elliptical to oblong and ovoid basidiospores, presence of pleurocystidia and subglobose, lageniform, cylindrical, or lanceolate caulocystidia (Song and Bau 2023). Similarly, *C. himalayana* a Chinese taxon, differs due to its decurved margins, subglobose, orange brown pileus with faintly pubescent surface, sometimes stipe forming a bulb, ovoid to ellipsoid basidiospores, lageniform cheilocystidia (Wang et al. 2024). Ecologically, *C. safariensis* is, terrestrial, grows under *Tamarix aphylla*, whereas its sister's species *C. ceracea* grows under potted Orchidaceae plants, and *C. himalayana* grows on grassland (Song and Bau 2023; Wang et al. 2024). With these additions, the taxa of *Conocybe* known from Pakistan turn out to be 16.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

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No ethical statement was reported.

Use of AI

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Conceptualization: MH, ARN, ANK, PL, YJ, QL. Data curation: MH. Formal analysis: PL, ANK, ARN, MH. Funding acquisition: QL, YJ. Investigation: ANK. Methodology: MH. Software: MH. Supervision: ARN, ANK, QL. Visualization: MH. Writing - original draft: MH. Writing - review and editing: YJ, ANK, QL, MH, PL.

Author ORCIDs

Muhammad Haqnawaz https://orcid.org/0000-0003-4667-242X
Abdul Rehman Niazi https://orcid.org/0000-0002-1118-1148
Abdul Nasir Khalid https://orcid.org/0000-0002-5635-8031
Qirui Li https://orcid.org/0000-0001-8735-2890

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

ITS sequences

Authors: Yilan Jin, Muhammad Haqnawaz, Abdul Rehman Niazi, Pingzhu Lu, Abdul Nasir Khalid, Qirui Li

Data type: docx

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Supplementary material 2

LSU sequences

Authors: Yilan Jin, Muhammad Haqnawaz, Abdul Rehman Niazi, Pingzhu Lu, Abdul Nasir Khalid, Qirui Li

Data type: fas

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